



CLEAN COPY OF REPLACEMENT PAGES 8 AND 9

membrane by means of direct transfer electrophoresis (DTE). The individual sequence leaders were successively decoded by successively hybridizing with specific oligonucleotides.

The specific conditions for the amplification were as follows:

Forward primer ADRBR-F1 with the sequence

5'- TATTGGCCAGGATCTTTGCTTCTAT-3' (SEQ ID No. 8) and backward primer ADRBR-R1 with the sequence 5'- TAACATTAAGAACATTTGAAGC -3' (SEQ ID No. 9) were used for fragment I. Fragment II was amplified by means of the two primers ADRBR-F2 5'- GCATACCCCGCTCCAGATAAA -3'(SEQ ID No. 10) and ADRBR-R2 5'- GCACGCACATACAGGCACAAATAC -3' (SEQ ID No. 11). For fragment III it were two primers ADRBR-F3: 5'- GGCGCGTTCTGTGTTGG -3' (SEQ ID No. 12) and

E¹
ADRBR-R3: 5'- AGTGC GTTCTGCCGTTATGTG -3' (SEQ ID No. 13). For fragment VIII the two primers ADRBR-F8: 5'- GGTACTGTGCCTAGCGATAAC -3' (SEQ ID No. 14) and ADRBR-R8: 5'- TAAAATACCCGTGTGAGCAAATAAGAG -3' (SEQ ID No. 15) were used. The reactions conditions for these four fragments were as follows: 10 x PCR buffer (100 nM Tris HCl, 15 mM MgCl₂ x 6H₂O, 500 mM KCl, pH 8.3), dNTP 2 mM, 30 μM primer F, 30 μM primer R, 50 ng of genomic DNA and 5 U of a *Taq* DNA polymerase. All three fragments were amplified with the following temperature profile: 94°C 4 min; 35 cycles: 94°C 30 sec., 60°C 30 sec., 72°C 1 min. and finally 72°C 10 min.

Fragment IV was amplified with the aid of the two primers ADRBR-F4:

5'- GGGGAGGGAAAGGGGAGGGAG -3' (SEQ ID No. 16) and ADRBR-R4:

5'- CTGCCAGGCCATGACCAGAT -3' (SEQ ID No. 17). For fragment VII the primers

ADRBR-F7: 5'- CTGGCTGCCCTTCTTCATCGTT -3' (SEQ ID No. 18) and ADRBR-R7: 5'- TACCCTAAGTTAAATAGTCTGTT -3'(SEQ ID No. 19) were used. The conditions for these two PCR reactions were as follows: 10 x PCR buffer (160 mM (NH₄)₂SO₄, 0.1% of Tween-20, 500 mM KOH, pH), dNTP 2 mM, 30 μM primer F, 30

μ M primer R, 50 nG of genomic DNA and 4 U of a mixture of *Taq* DNA polymerase and a thermostable inorganic pyrophosphatase of *thermos thermophilus*. Both fragments were amplified with the following temperature profile: 94°C 4 min.; 35 cycles: 94°C 30 sec., 66°C [fragment IV] or 60°C [fragment VII] 30 sec., 72°C 1 min. and finally 72°C 10 min.

Fragment V was amplified by means of the two primers

ADRBR-F5: 5'- ATGCCCGGACACGAC -3' (SEQ ID No. 20) and

ADRBR-R5: 5'- GTAGAAGGACACGATGGA -3' (SEQ ID No. 21), fragment VI was amplified with the two primers

ADRBR-R6: 5'- GCTACTTGCCATTACTTCACC -3' (SEQ ID No. 22) and

ADRBR-R6: 5'- AAATCTGGGCTCCGGCAGTAGATAAG -3' (SEQ ID No. 23).

E
Cont.
These two fragments were amplified by means of 'AmpliTaq gold kits' by Perkin Elmer. In these two fragments the temperature profile was as follows: 94°C 10 min.; 35 cycles: 94°C 30 sec., 56°C [fragment V] or 58°C [fragment VI] 30 sec., 72°C 1 min. and finally 72°C 10 min.

Sequencing was carried out by means of the 'thermo sequenase cycle sequencing kit' by Amersham. The PCR primers described above were used as sequencing primers.

ADRBR-F1, ADRBR-F3, ADRBR-F5 and ADRBR-R7. Fragments I, III, V and VII were inserted into the two sequencing pools. Yet, pool 3 contained the sequencing primers ADRBR-F2, F4, F6 and F8; pool 4 contained the sequencing primers ADRBR-R2, R4, R6 and R8. Fragments II, IV, VI, VII were inserted into these two pools.

All PCR and sequencing reactions were carried out in a PTC 225 cycler of MJ Research.

The products of the sequencing reaction were separated on a 100 μ m thick acryl amide gel (5% acryl amide, 7 M urea) and under standard DTE conditions (see Richterich and Church, 1993) transferred to a biodyne A membrane (Pall). Then, the membrane was hybridized with 32 P-marked oligonucleotides and the individual sequence leaders were detected with the aid of a phosphor fluorimager (Storm 860, Molecular Dynamics).